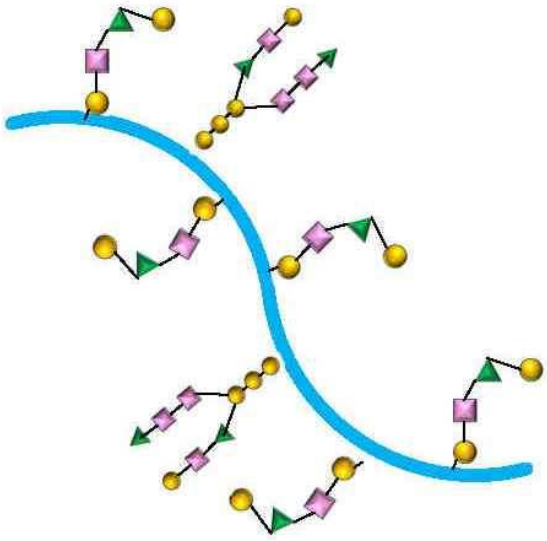

GLYCANS: COVID AND CANCER

TGL 190

ABSTRACT
This Report examine glycans, sugar complexes, on surface proteins and their impact on disease. This is a high level Report that presents and discusses the issue of glycans. We examine recent work of glycan impact on COVID as well as ongoing efforts understanding the impact on a variety of cancers.

Terrence McGarty
December 2021

Notice

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Contents

1 INTRODUCTION

To paraphrase the Roman writer Phaedrus, “nothing is necessarily what it appears to be”. We all too often create paradigms that we can become fixated upon and then become frustrated when we cannot explain the variances in subsequent experiments. For example, we know that certain cell receptors, proteins, can be activated and result in certain pathological states. If we can block them we can prevent the pathology. But frequently we can achieve that in some but not all patients.

We may wonder why and yet be stuck in the paradigm we have set out for ourselves. Looking deeper we may see that the glycosylation of the protein may dramatically alter its binding capability for better or worse. This then shifts the paradigm, a change all too often difficult to make. Herein we examine glycans and their functions. Our intent is not to provide any definitive work on glycosylation but to just open the widow and take a glance at an extended paradigm of cellular control.

Much of the current paradigms for such illnesses as cancer and COVID look at ligands and receptor proteins in what can best be called a naked presentation. Namely one sees the protein unfettered by any other obstacles. However, it has been well known that proteins, especially those external to a cell can collect a covering of sugar type molecules, often in long complex strands. They may almost appear as hair like elements along the surface of the protein. These molecules result in a process of glycosylation, and as such can often dramatically change the surface binding properties.

Herein we examine some recent studies of glycosylation in the context of COVID and cancers. In the case of COVID it is the glycosylation of the ACE2 receptor and the impact that such a process may have,

As Chatterjee and Saha have noted:

Protein glycosylation includes the addition of N-linked glycans, O-linked glycans, phosphorylated glycans, glycosaminoglycans, and glycosylphosphatidylinositol (GPI) anchors to amide backbones similarly to C-mannosylation of essential amino acid residues. Glycolipids are glycoconjugate which include glycosphingolipids (GSLs) formed through the addition of sugars to lipids.

Glycosylation of proteins and lipids happens within the endoplasmic reticulum (ER) and with most of the terminal processing occurring within the cis-, medial- and trans-Golgi compartments. In these organelles, glycosidases, and glycosyltransferases form carbohydrate structures in a series of steps that are dominance by the availability of the enzyme activity, substrate, levels of gene transcription, and enzyme location.

In fact, the glycome of a specific cell reflects its distinctive gene-expression pattern that controls the level of the enzymes responsible for glycoconjugation

As Varki had noted:

Simple and complex carbohydrates (glycans) have long been known to play major metabolic, structural and physical roles in biological systems. Targeted microbial binding to host glycans has also been studied for decades. But such biological roles can only explain some of the remarkable complexity and organismal diversity of glycans in nature. Reviewing the subject about two decades ago, one could find very few clear-cut instances of glycan-recognition-specific biological roles of glycans that were of intrinsic value to the organism expressing them. In striking contrast there is now a profusion of examples, such that this updated review cannot be comprehensive.

Instead, a historical overview is presented, broad principles outlined and a few examples cited, representing diverse types of roles, mediated by various glycan classes, in different evolutionary lineages. What remains unchanged is the fact that while all theories regarding biological roles of glycans are supported by compelling evidence, exceptions to each can be found. In retrospect, this is not surprising.

Complex and diverse glycans appear to be ubiquitous to all cells in nature, and essential to all life forms. Thus, >3 billion years of evolution consistently generated organisms that use these molecules for many key biological roles, even while sometimes coopting them for minor functions. In this respect, glycans are no different from other major macromolecular building blocks of life (nucleic acids, proteins and lipids), simply more rapidly evolving and complex. It is time for the diverse functional roles of glycans to be fully incorporated into the mainstream of biological sciences

The objectives of this Report are as follows:

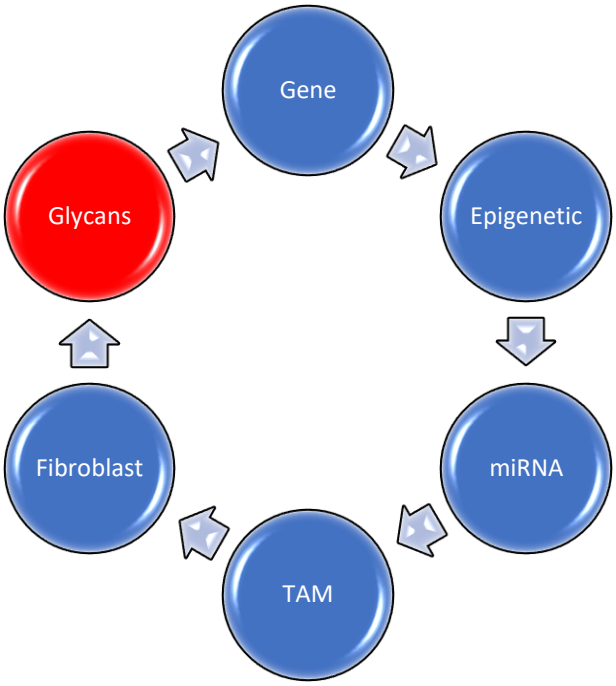
1. Present the elements of glycans and their relationship to proteins.

2. Discuss some of the current understanding of how glycans effect disease states. We use recent studies on the COVID virus and then we also examine the impact of glycans on a variety of cancers.

3. This is not a definitive study of glycans. Such has been done by many of the authors referenced herein. This is however a presentation of another element in the development and expansion of cell control mechanisms especially those related to cancers.

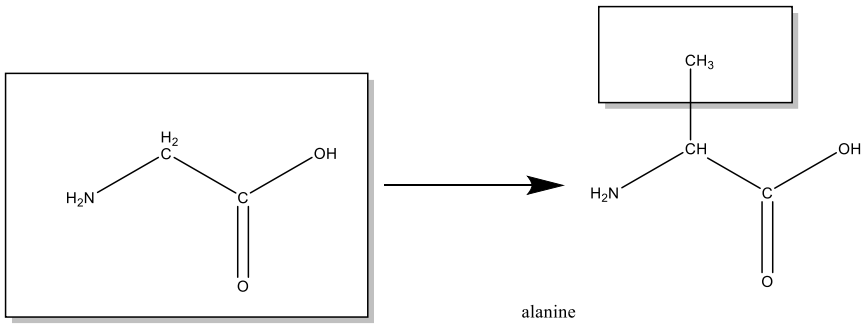
We all too often see a focus on one specific pathway or even more closely one specific gene or protein. Cancer is a highly multifaceted organ disease ranging from epigenetic, genetic, local and distant environments as well as the impact of such factors as glycosylation.

We can envision the multiple complexities in the graphic below. Cancers have many drivers. From the basic genetic alterations, thus producing aberrant mRNA and proteins, through epigenetic changes, micro RNAs. tumor associate immune cells such as macrophages, fibroblasts and associated stroma, and glycans and associated protein modifications. In this Report our focus is on the glycan leg of this process.


Gene
Epigenetic
miRNA
TAM
Fibroblast
Glycans

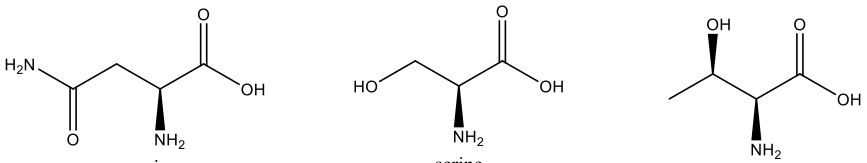
2 AMINO ACIDS

Let us begin with a brief review of some basics on amino acids. The basic examples are below. They have an N terminal and an OH terminal, also the O terminal. Connecting a specific R (carbon group) collection results on a specific amino acid. The amino acids then bond from O to N and from that we obtain a complete protein formation.



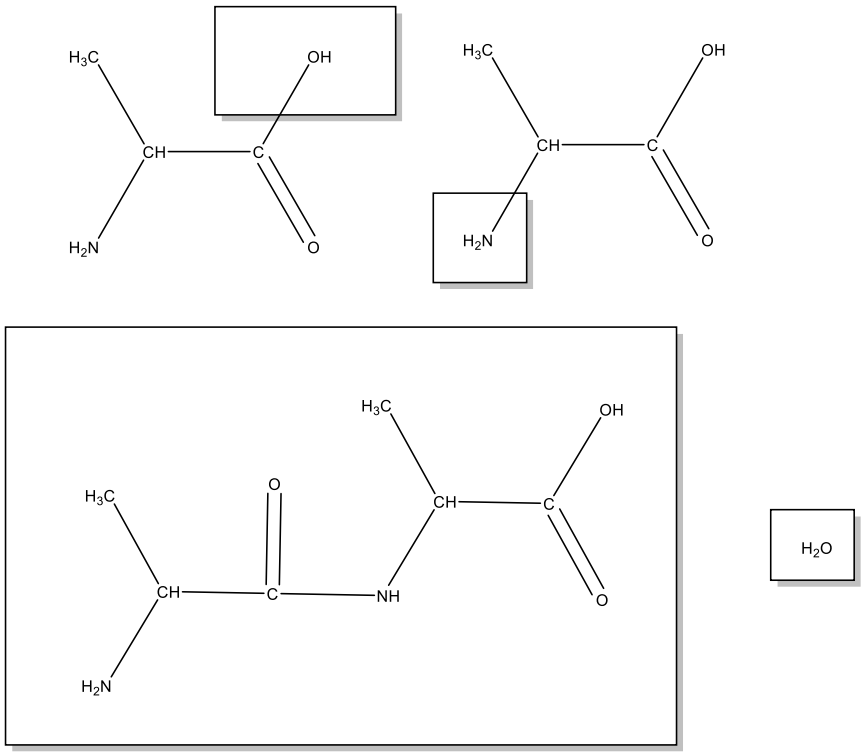
glycine alanine

The three amino acids that we focus on in the discussion of glycans are asparagine, serine and threonine. We also show them below as well. From the perspective of glycans and protein implications these are the three of interest.

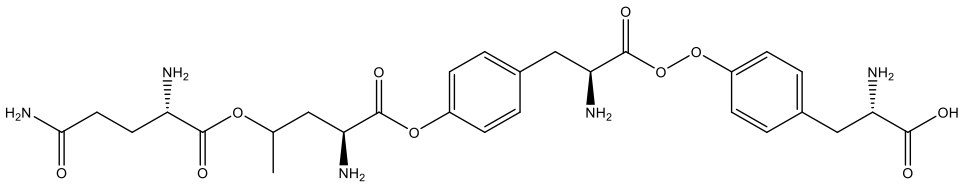


asparagine serine threonine

Now we show below the typical process of binding of the amino acids together into a protein element. The N bonds with the O on the OH and releases a water molecule which we show below.



Now a longer collection of amino acids is shown below. It is to this string that we will see the attachment of glycans or sugar molecules.



Many tend to think of proteins as just a collection of amino acids folded in a complex manner. Namely if a protein is N amino acids in length, then any matching protein may very well be like any other matching protein. However, if the protein is glycosylated, then one glycosylation may differ from another and the underlying protein may have a materially different impact. Thus glycans can in a sense be the miRNAs of proteins.

For a reminder, the Table below presents the list of twenty amino acids found in humans. For glycosylated proteins we focus on just three.

Amino acid	Abbreviation	Single letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I

Amino acid	Abbreviation	Single letter abbreviation
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

3 CARBOHYDRATES

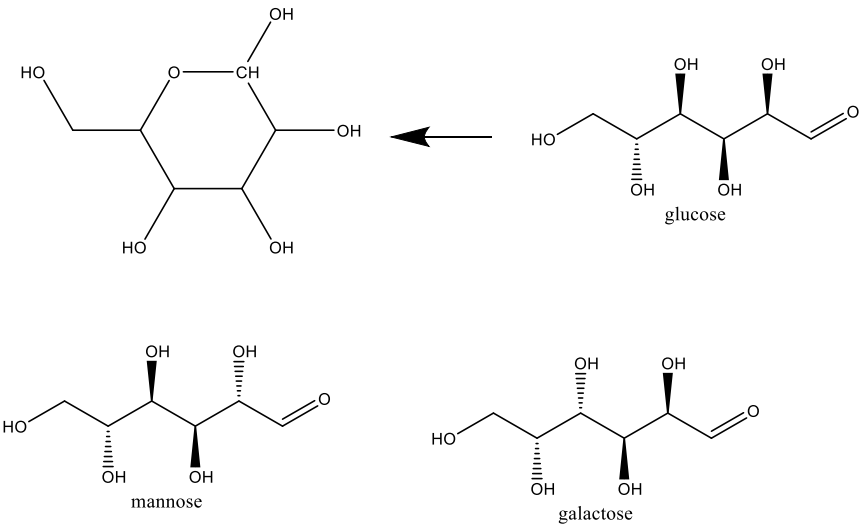
To understand glycans we must understand amino acids and carbohydrates. Here we briefly review some of the key issue with the carbohydrates in this area.

As Varki and Kornfield have noted (see Varki et al):

…glycobiology is the study of the structure, biosynthesis, biology and evolution of saccharides (…carbohydrates, sugars, …or glycans) that are widely distributed in nature and the proteins that recognize them.

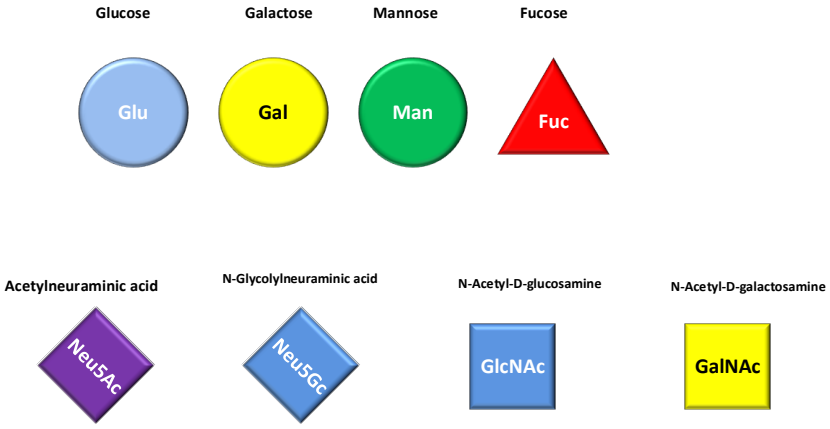
Simply put for our purposes, it is the focus on the interaction of sugars and proteins and particularly focusing on proteins involved in cellular signalling of all sorts.

In the context of our focus there are three sugars we examine. They are glucose, mannose and galactose. Below we review their linear form and their cyclic form.



A sample of some of the operative sugars we are concerned with are shown below is what is considered a set of canonical structures. Glucose is the most well-known but there are many other structures displayed. These sugars can be covalently bonded to create strings or even trees of such sugars. We shall show this in the next section. The graphic below is from KEGG database and presents the generally accepted set of glycans and their representations. We shall adhere to this form mostly.

https://www.genome.jp/kegg/catalog/codes2.html



The above eight glycans represent the set of most common glycans. They can then be attached to one another which we shall discuss in the next section.

4 GLYCANS

Glycans and glycosylation are basically the attachment of sugar molecules, often in linkages of multiple basic sugars, to another molecule. In the case at point, we examine these attached sugars in terms of proteins.

4.1 INTRODUCTION

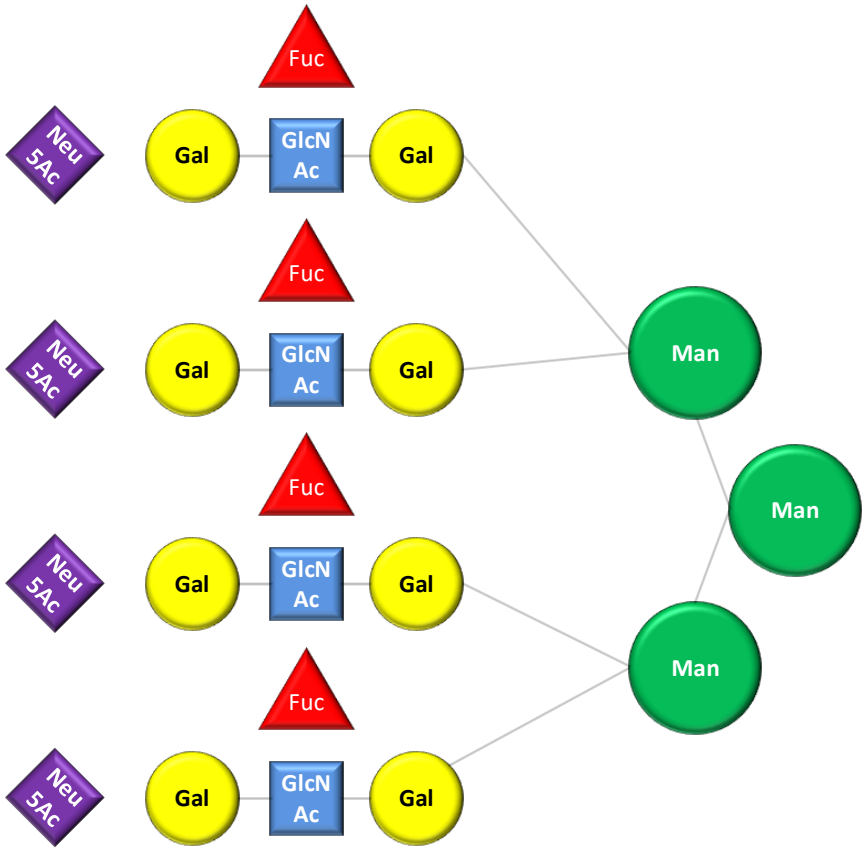
Varki has presented a significant update regarding glycans. He notes:

Simple and complex carbohydrates (glycans) have long been known to play major metabolic, structural and physical roles in biological systems. Targeted microbial binding to host glycans has also been studied for decades. But such biological roles can only explain some of the remarkable complexity and organismal diversity of glycans in nature.

Reviewing the subject about two decades ago, one could find very few clear-cut instances of glycan-recognition-specific biological roles of glycans that were of intrinsic value to the organism expressing them. In striking contrast there is now a profusion of examples, such that this updated review cannot be comprehensive. Instead, a historical overview is presented, broad principles outlined and a few examples cited, representing diverse types of roles, mediated by various glycan classes, in different evolutionary lineages.

What remains unchanged is the fact that while all theories regarding biological roles of glycans are supported by compelling evidence, exceptions to each can be found. In retrospect, this is not surprising. Complex and diverse glycans appear to be ubiquitous to all cells in nature, and essential to all life forms. Thus, >3 billion years of evolution consistently generated organisms that use these molecules for many key biological roles, even while sometimes coopting them for minor functions. In this respect, glycans are no different from other major macromolecular building blocks of life (nucleic acids, proteins and lipids), simply more rapidly evolving and complex. It is time for the diverse functional roles of glycans to be fully incorporated into the mainstream of biological sciences.

Below we show a tree like glycan structure which has the ability to link itself to a protein. There is a mannose base and then four identical branches of linked sugars.

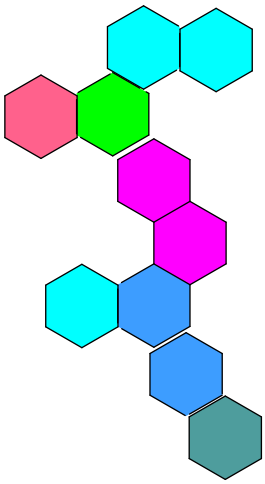


As Bao et al have noted:

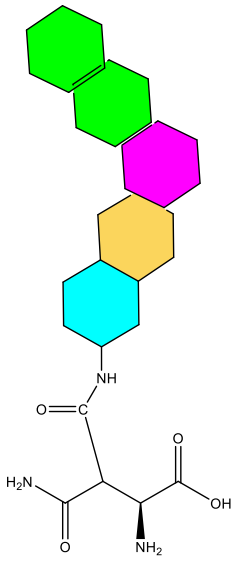
Glycosylation is a complex post-translational modi and it decorates one- proteins. Glycans account for a fifth to one-half of eukaryotic –25% of dry cell mass frcation and have essential functional and pathological roles. Despite their importance, glycans have complex structures that are difficult to study. The complex structures of glycans arise from a context-sensitive biosynthetic network involving dozens of enzymes.

A simple change of a single intermediate glycan or glycosyltransferase will have cascading impacts on the glycans secreted. Unfortunately, current data analysis approaches for glycoprofiling and glycomic data lack the critical systems perspective to decode the interdependence of glycans easily. It is important to understand the network behind the glycoprofiles to understand the behavior of the process better.

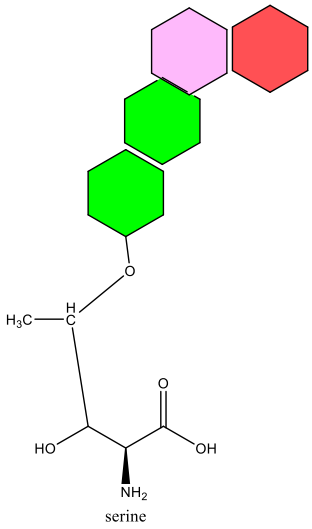
Another view is to see a collection of sugars as shown graphically below. They may be a disparate variety of the ones we have already introduced. They become bound together to effect a complex glycan appendage to a protein.



Now these glycans become attached in two generally specific manners. One is an attachment via the N on the amino acid and the other the O. Then the N and O types bind to the respective amino acids as shown below.

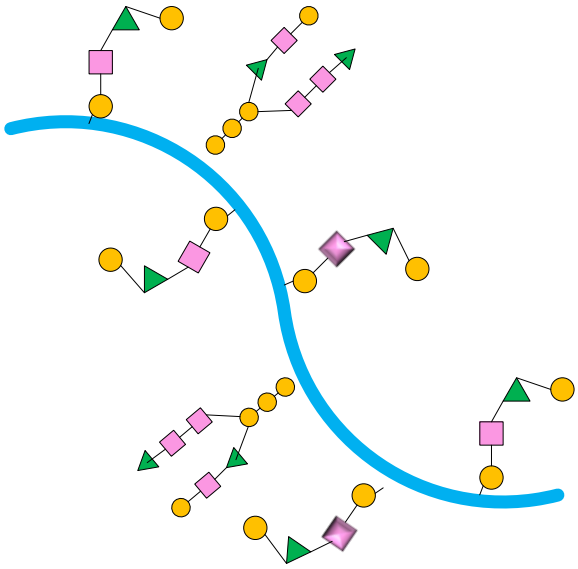


asparagine
N linked



serine
O linked

Subsequently one can imagine a multiplicity of such bindings along the entire length of the protein appearing as “hairs” bristling off the folded protein.



This collection of glycans can dramatically change the binding characteristics of the protein. Recall that all the sugars have a multiplicity of oxygen atoms which can create charge displacements.

Now as Koropatkin et al have noted:

Symbiotic microorganisms that reside in the human intestine are adept at foraging glycans and polysaccharides, including those in dietary plants (starch, hemicellulose and pectin), animal-derived cartilage and tissue (glycosaminoglycans and N-linked glycans), and host mucus (O-linked glycans).

Fluctuations in the abundance of dietary and endogenous glycans, combined with the immense chemical variation among these molecules, create a dynamic and heterogeneous environment in which gut microorganisms proliferate*. In this Review, we describe how glycans shape the composition of the gut microbiota over various periods of time, the mechanisms by which individual microorganisms degrade these glycans, and potential opportunities to intentionally influence this ecosystem for better health and nutrition.*

The above does raise the issue of how the glycans are generated. Are they driven by diet, are they organ specific, are the genetically driven? There has been some reasonable work along the lines of addressing these questions but there still remains a great deal of unknown.

4.2 N LINKED

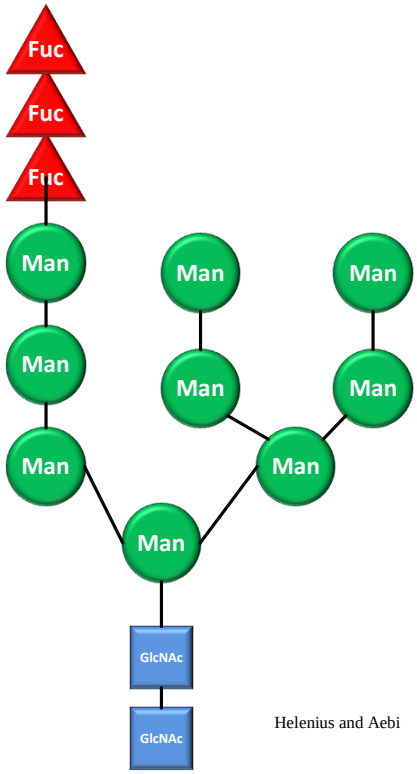
The N linked versions attach specifically to **asparagine**. We have shown this previously. As Helenius and Aebi have noted:

N-linked oligosaccharides arise when blocks of 14 sugars are added cotranslationally to newly synthesized polypeptides in the endoplasmic reticulum (ER). These glycans are then subjected to extensive modification as the glycoproteins mature and move through the ER via the Golgi complex to their final destinations inside and outside the cell. In the ER and in the early secretory pathway, where the repertoire of oligosaccharide structures is still rather small, the glycans play a pivotal role in protein folding, oligomerization, quality control, sorting, and transport.

The above describes the intracellular processes involved in the generation of the glycan associated proteins.

They are used as universal "tags" that allow specific lectins and modifying enzymes to establish order among the diversity of maturing glycoproteins. In the Golgi complex, the glycans acquire more complex structures and a new set of functions. The division of synthesis and processing between the ER (endoplasmic reticulum) and the Golgi complex represents an evolutionary adaptation that allows efficient exploitation of the potential of oligosaccharides.

The authors (Helenius and Aebi) demonstrate several forms and a specific N linked core is presented as follows:



Helenius and Aebi

Note the significant complex structure of this glycan. It is important to also note that such complexity could dramatically change the protein binding and conformation. Where they note:

The N-linked core oligosaccharide. N-linked glycans are added to proteins in the ER as "core oligosaccharides" that have the structure shown. ***These are bound to the polypeptide chain through an N-glycosidic bond with the side chain of an asparagine that is part of the Asn-X-Ser/Thr consensus sequence****. Terminal glucose and mannose residues are removed in the ER by glucosidases and mannosidases.*

The authors continue:

In mature glycoproteins, N-linked glycan moieties are structurally diverse. The sugar composition and the number and size of branches in the sugar tree varies among glycans bound to a protein, among glycoproteins, and among cell types, tissues, and species. However, when initially added in the ER to growing nascent polypeptides, the glycans do not display such heterogeneity. The "core glycans" are homogeneous and relatively simple.

Indeed the complexity of the associated glycans is a driving factor for their impact. Moreover the actual process of creating the complex glycan mix appears as of yet undetermined.

The trimming and processing that the glycans undergo when the glycoprotein is still in the ER introduce only limited additional diversity, because the alterations are shared by all glycoproteins. Thus, the spectrum of glycoforms remains rather uniform until the glycoproteins reach the medial stacks of the Golgi apparatus, where structural diversification is introduced through a series of nonuniform modifications.

Particularly in vertebrate and plant cells, it is the terminal glycosylation in the Golgi complex that gives rise to the tremendous diversity seen in glycoconjugates that reach the cell surface. The switch from structural uniformity in the ER to diversification in the Golgi complex coincides with a marked change in glycan function. In the early secretory pathway, the glycans have a common role in promoting protein folding, quality control, and certain sorting events.

Later, Golgi enzymes prepare them for the spectrum of novel functions that the sugars display in the mature proteins. Here, we mainly address events in the early secretory pathway. We focus on observations that are starting to unmask the logic of the various early trimming and modification events. We also discuss glycan structure and function in light of fundamental differences between the two biosynthetic organelles, the ER and the Golgi complex.

Thus, the oligosaccharide as an N linked version would then attach to an asparagine. As Pearce notes:

N-glycosylation follows a strictly ordered assembly, and the site of modification is predictable to ***asparagine residues*** *(N) of a peptide/ protein only when an NXT/S sequon is present (where X is any residue accept proline). There are two major changes that can occur to the core N-glycan structure, which are increased frequency of a bisecting GlcNAc, or β1,6 and β1,4 branching to the core pentasaccharide.*

Other notable changes occur to the epitopes of secondary structures that are attached to the core N-glycan structure, namely the N-acetyllactosamine units and their further functionalizations (discussed under Cancer epitopes within structures common to different core glycans).

Additionally, whilst O-glycan epitopes are usually discussed as distinct disease specific epitopes, N-glycans tend to be discussed in terms of a change to the pattern of the N-glycome.

In other words, the structures identified are synthesized in normal tissues, but the pattern is altered in disease. Below the bisecting GlcNAc and branching core N-glycome patterns are discussed, followed by other changes in the pattern of the N-glycome in various cancers. Specific epitope changes to N-acetyllactosamines on N-glycans are covered later, as mentioned earlier

N linked glycans play a significant role in receptor functions. However due to their significant diversity and presence understanding that is a challenge.

4.3 O LINKED

Now we briefly examine the other glycan, O linked glycans. As Pearce notes:

*O-GalNAc glycans are attached to proteins at **serine or threonine** sites. Although there is no defined sequon where an O-glycan is attached, they are often found within variable number of tandem repeat (VNTR) domains, which are high in serine and threonine repeats.*

On a mucin, hundreds of O-glycan's can be present within the VNTR regions, expressed in a variety of glycoforms. Mucins are produced primarily by epithelial cells on the surfaces of various membranes, and secreted into the extracellular space. In healthy cells mucins are presented on the apical surface, but cells loose this polarization during malignant transformation, which supports an invasive phenotype (for background information and further reading on this phenomenon see elsewhere.

For example, membrane type I matrix metalloproteinase (MT1-MMP) polarization in malignant transformation is lost on the apical surface of epithelial cells and is found to concentrate in specific membrane structures that sit close to the basement membrane, and aid invasion through degradation of the basement membrane, and activation of other MMPs which are capable of degrading the collagen rich extracellular matrix that often surrounds malignant cells.

The first step in O-glycan (O-GalNAc) synthesis is UDPGalNAc transferred to a Ser/Thr by ppGalNAcTs, a family of enzymes consisting of ~20 member. O-glycan's are characterized across eight core structures.

Overexpression and/or aberrant expression of mucins by carcinomas has been known for many years. In general, mucins act as anti-adhesins, and therefore aid displacement of malignant cells during metastasis.

The last observation is important since it focuses on the metastatic behavior. We often look at surface proteins such as E cadherin and its change to N cadherin as a driving metastatic factor.

However, the glycan function and the mucin production add an additional element worthy of note. Thus understanding glycans in the context of a malignant lesion appears to be as critical as almost all other factors.

5 COVID

Recent work has demonstrated the impact of glycans on various parts of the COVID process. We examine some of this in this section.

5.1 COVID BASICS

COVID

5.1.1 Infection

Infection with the Corona virus is through nasal passages of the nasopharynx. It results from aerosol particles from an infected person or through contact with surfaces infected by similar aerosols. Many of the specifics of the infection process are still unknown. One suspects that it is based upon aerosols. But there is still no dispositive scientific evidence, just decades of anecdotal experience.

Along comes the CDC. They now state[1]:

COVID-19 is thought to spread mainly through close contact from person-to-person. Some people without symptoms may be able to spread the virus. We are still learning about how the virus spreads and the severity of illness it causes.

Person-to-person spread: The virus is thought to spread mainly from person-to-person.

1. Between people who are in close contact with one another (within about 6 feet).

2. Through respiratory droplets produced when an infected person coughs, sneezes, or talks.

3. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.

4. COVID-19 may be spread by people who are not showing symptoms.

The virus spreads easily between people: How easily a virus spreads from person-to-person can vary. Some viruses are highly contagious, like measles, while other viruses do not spread as easily. Another factor is whether the spread is sustained, which means it goes from person-to-person without stopping. The virus that causes COVID-19 is spreading very easily and sustainably between people. Information from the ongoing COVID-19 pandemic suggests that this virus is spreading more efficiently than influenza, but not as efficiently as measles, which is highly contagious. In general, the more closely a person interacts with others and the longer that interaction, the higher the risk of COVID-19 spread.

[1] https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html

The virus may be spread in other ways: It may be possible that a person can get COVID-19 by touching a surface or object that has the virus on it and then touching their own mouth, nose, or possibly their eyes. This is not thought to be the main way the virus spreads, but we are still learning more about how this virus spreads.

This is what we said in early March and what my grandmother said in 1945! This is the nth revision of the CDC assessment. There seems to be a serious and chronic problem there. One wonders when it can and must be corrected?

As an added note, as we had also discussed, the virion travels in an encased aerosol, namely an air-filled water encased bubble that moves about with Newtons laws of motion as well as following Archimedes principle by floating. The aerosol also has thermodynamic effects as well with Brownian motion effects resulting from random collisions[2]. Thus aerosols "linger" depending upon a multiplicity of factors. Just what makes for lingering aerosols depends. There is a significant body of knowledge in atmospheric aerosols but virion containing ones, not so much.

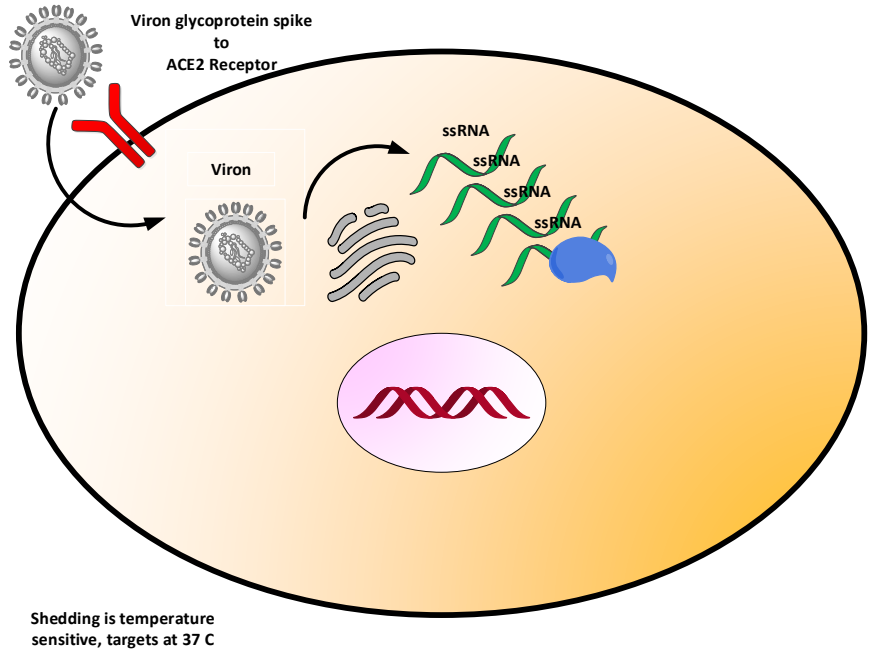
What is lacking is good quality physical and clinical research. Unfortunately, the CDC does not seem as advance as my grandmother back in 1918.

5.1.2 Corona Specifics

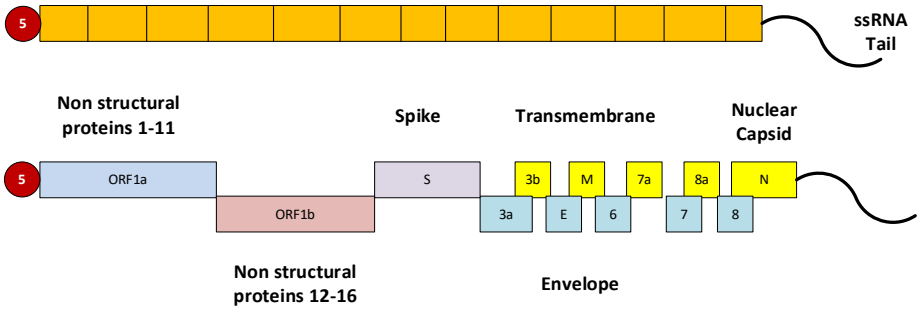
Let us now examine the specifics of corona. Corona are large positive single strand RNA viruses with surface ligands that bind to ACE2 receptors on epithelial cells and then progress to multiply internally at a temperature of 37 C. Unlike rhinoviruses which are epithelial but multiply at 35 C, the nasal passageways, the corona needs to move to the lungs and the higher temperature to fully expand.

The figure below summarizes this virus. The RNA is about 30,000 base pairs and when translated can produce eight operable regions for the generation of proteins or RNA replication.

[2] see Seinfeld and Pandis, Atmospheric Chemistry and Physics, Wiley, 2016, Chapters 9-10



These regions of the RNA of the Corona virus are depicted below. First the ssRNA is combines with it complement creating a double strand and within that double strand we have sub units which will give rise to the protein elements necessary for its replication. Key to that will be a polymerase allowing for the production of the elements.



The details of these eight elements are shown as below. The RNA has a protein on one end and a tail at the other end. The eight active sections are depicted including the two replicase regions essential for reproducing the ssRNA and other smaller segments involved in the process. There is a reasonable understanding as to the virologic processes associated with the COVID virion. The replication of the virus is described by Oxford et al (5th Ed):

Virions initially attach to the cell plasma membrane through specific receptors. These have been identified for several coro-naviruses; for example, human coronavirus uses the membrane-bound metalloproteinase, aminopeptidase N (APN), whereas OC43 simply binds to sialic acid groups on cell-surface proteins. SARS CoV uses the host-cell receptor angiotensin-converting enzyme 2 (ACE2) to gain entry into cells whereas MERS CoV uses the host receptor dipeptidyl

peptidase 4 (DPP4). Uptake into cells is rapid and temperature-dependent, involving fusion with the plasma membrane or via endocytosis followed by a spike-mediated fusion in the endosome. Large multinucleated giant cells, syncytia, can be formed both in the laboratory and in an infected host.

Once released into the cytoplasm the virus positive-strand RNA is translated directly into two polypeptides: ORFla and ORFlb at the 5' end of the genome. These are processed to form a replicase-transcriptase complex that possesses RNA polymerase activity. The RNA polymerase transcribes a full-length negative RNA strand, which acts as the template for transcription of multiple subgenomic virus mRNAs.

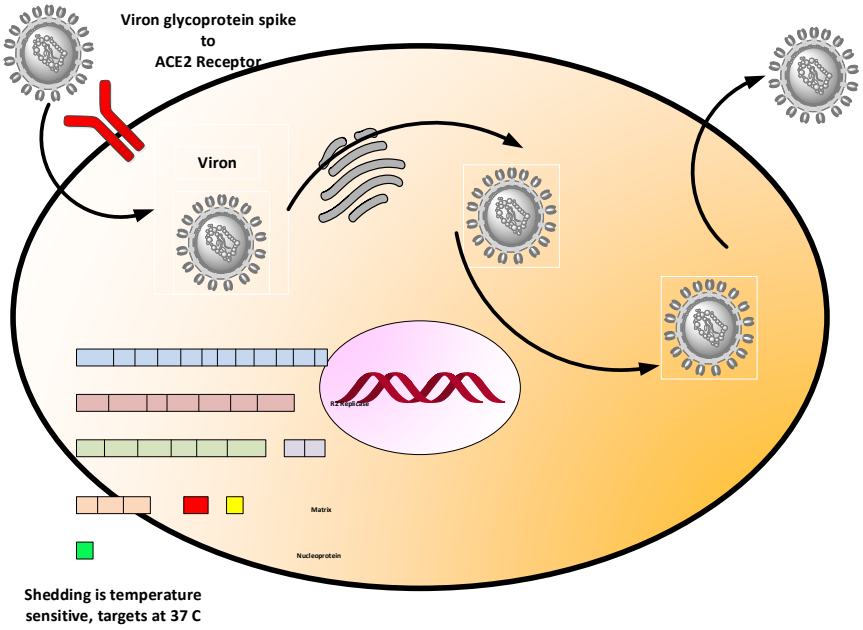
Coronavirus mRNAs are unusual in that they all terminate at the common 3' end of the genome, but start at various places from the 5' end to produce a nested set of 3' co-terminal transcripts. Each of the eight mRNAs, except for the smallest, therefore encode for multiple proteins, with the longest one being, in effect, full-length coronavirus genome RNA and the others in descending order of size being S, E, M, and N. Generally, each subgenomic virus mRNA is the template for translation into one protein. There are 16 non-structural proteins (l-16nsp), some of which have proteinase functions or are polymerases, including RNA-dependent RNA polymerase (nspl2) and endoribonuclease (nspl5).

Virus proteins that constitute the virus particle, namely N, M, and S, are produced in the infected cell and new virion assembly occurs initially in the cytoplasm on smooth-walled vesicles located between the ER and the Golgi known as ERGIC (endoplasmic reticulum Golgi intermediate compartment).

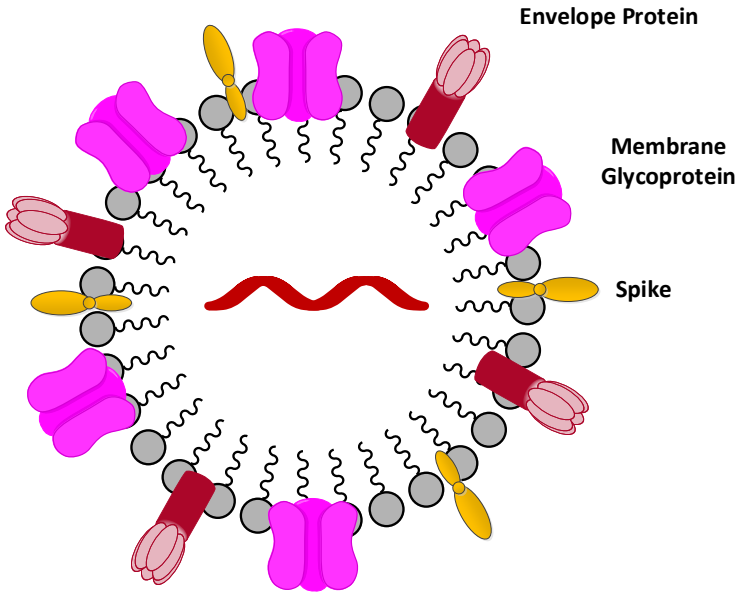
There newly formed RNP interacts with the M protein from the ER, and M interacts with the S and other proteins to form the infectious virus which buds into the Golgi, thereby acquiring a lipid envelope. Envelope proteins are glycosylated in the Golgi. Virions are released by fusion of smooth-walled virion-containing vesicles with the plasma membrane. As with other RNA viruses, the lack of proofreading functions in the virus RNA polymerase leads to a high rate of mutation in the new virus genomes.

The very long genomes, together with the discontinuous RNA replication, can favour recombination leading to new genotypes with varying pathogenicity. There remains also the possibility of recombination between zoonotic corona viruses and between human viruses. Recombination can allow corona viruses to rapidly evolve and adapt to new ecological niches.

We demonstrate the above in the figure below:



The details of the virion appear below with the prominent Spike protein on the surface surrounded by a lipid layer. The single stranded RNA is contained inside.



As we will note, the above structure becomes the target for a vaccine development. Specifically, the unique surface proteins. As we shall note, the spike protein is specific to this virus and if we can then design an antibody to attack that site then we can utilize the immune system itself.

5.2 GLYCANS AND RECEPTOR CONTROL

Recent work focusing on the receptor control in the COVID corona virus as illuminated the role of glycans. As Sztain et al note :

*SARS-CoV-2 infection is controlled by the **opening of the spike protein receptor binding domain (RBD), which transitions from a glycan-shielded 'down' to an exposed 'up' state to bind the human angiotensin-converting enzyme 2 receptor and infect cells**.*

While snapshots of the 'up' and 'down' states have been obtained by cryo-electron microscopy and cryo-electron tomagraphy, details of the RBD-opening transition evade experimental characterization. Here over 130 μs of weighted ensemble simulations of the fully glycosylated spike ectodomain allow us to characterize more than 300 continuous, kinetically unbiased RBD-opening pathways.

*Together with ManifoldEM analysis of cryo-electron microscopy data and biolayer interferometry experiments**, we reveal a gating role for the N-glycan at position N343, which facilitates RBD opening.** Residues D405, R408 and D427 also participate. The atomic-level characterization of the glycosylated spike activation mechanism provided herein represents a landmark study for ensemble pathway simulations and offers a foundation for understanding the fundamental mechanisms of SARS-CoV-2 viral entry and infection.*

As Hsu et al have noted:

Glycans of the SARS-CoV-2 spike protein are speculated to play functional roles in the infection processes as they extensively cover the protein surface and are highly conserved across the variants. To date, the spike protein has become the principal target for vaccine and therapeutic development while the exact effects of its glycosylation remain elusive.

Experimental reports have described the heterogeneity of the spike protein glycosylation profile. Subsequent molecular simulation studies provided a knowledge basis of the glycan functions. However, there are no studies to date on the role of discrete glycoforms on the spike protein pathobiology. Building an understanding of its role in SARS-CoV-2 is important as we continue to develop effective medicines and vaccines to combat the disease.

*Herein, we used designed combinations of glycoengineering enzymes to simplify and control the glycosylation profile of the spike protein **receptor-binding domain (RBD**). Measurements of the receptor binding affinity revealed the regulatory effects of the RBD glycans. Remarkably, opposite effects were observed from differently remodeled glycans, which presents a potential strategy for modulating the spike protein behaviors through glycoengineering.*

Moreover, we found that the reported anti-SARS-CoV-(2) antibody, S309, neutralizes the impact of different RBD glycoforms on the receptor binding affinity. Overall, this work reports the regulatory roles that glycosylation plays in the interaction between the viral spike protein and host receptor, providing new insights into the nature of SARS-CoV-2. Beyond this study,

enzymatic remodeling of glycosylation offers the opportunity to understand the fundamental role of specific glycoforms on glycoconjugates across molecular biology.

Hsu et al continue by describing modelling of the binding energy and the glycan impact:

In addition to the S protein RBD, ACE2 is also a heavily glycosylated protein. Both theRBD glycan at N343 (N343RBD) and the ACE2 glycan at N322 (N322ACE2) are close to the binding interface with a distance of only 31.3 Å between the two glycans…

Modeling analysis indicated that these interactions generate 250-360 kJ/mol binding force, which contributes to about one-third of the RBD-ACE2 binding energy (890-1040 kJ/mol).

In other words, the RBD glycans interact with ACE2, which explains why remodeling their structures led to different binding affinities.

To examine this explanation, we measured the ACE2 binding affinity of deglycosylated RBD prepared by endoglycosidase F2 treatment. An apparent reduction in binding response was found with a KD value of 110 ± 3.3 nM, a weaker affinity than that of native RBD. Moreover, when the ACE2 N-glycans were removed, a dramatic decrease of binding affinity was observed. In combination with the published simulation study, our result suggested that the RBD glycans can stabilize ACE2 binding, likely through the interactions with the N322ACE2 glycans or the ACE2 backbone..

Lastly, as Nguyen et al note :

COVID-19 is a highly infectious respiratory disease caused by the novel coronavirus SARS-CoV-2. It has become a global pandemic and its frequent mutations may pose new challenges for vaccine design.

During viral infection, the Spike RBD of SARS-CoV-2 binds the human host cell receptor ACE2, enabling the virus to enter the host cell. Both the Spike and ACE2 are densely glycosylated, and it is unclear how distinctive glycan types may modulate the interaction of RBD and ACE2. Detailed understanding of these determinants is key for the development of novel therapeutic strategies. To this end, we perform extensive all-atom simulations of the (i) RBD-ACE2 complex without glycans, (ii) RBD-ACE2 with oligomannose MAN9 glycans in ACE2, and (iii) RBDACE2 with complex FA2 glycans in ACE2.

These simulations identify the key residues at the RBDACE2 interface that form contacts with higher probabilities, thus providing a quantitative evaluation that complements recent structural studies.

Notably, we find that this RBD-ACE2 contact signature is not altered by the presence of different glycoforms, suggesting that RBD-ACE2 interaction is robust.

This last above observation appears to indicate that glycans can have a minimal net effect. One suspects that significantly more work is required.

Applying our simulated results, we illustrate how the recently prevalent N501Y mutation may alter specific interactions with host ACE2 that facilitate the virus-host binding. Furthermore, our simulations reveal how the glycan on Asn90 of ACE2 can play a distinct role in the binding and unbinding of RBD. Finally, an energetics analysis shows that MAN9 glycans on ACE2 decrease RBD-ACE2 affinity, while FA2 glycans lead to enhanced binding of the complex.

Together, our results provide a more comprehensive picture of the detailed interplay between virus and human receptor, which is much needed for the discovery of effective treatments that aim at modulating the physical-chemical properties of this virus …

Since RBD binding to ACE2 is key to viral entry, this interaction is a major target for development of antibody therapeutics and vaccine design.

Mutations in the RBD can alter virus-receptor interaction, and thus, viral infectivity.

Recent structural studies have revealed the residue-residue contacts between SARS-CoV-2 RBD and ACE2, suggesting possible interactions that may determine the stability of the complex [5–7]. However, from these static data, it is unclear which of these residues are the critical ones that form interactions most frequently. Further, both the viral Spike and host receptor ACE2 are densely glycosylated with asparagine linked N-glycans [8,9].

Since some of these glycans are spatially located near the RBD-ACE2 interface, it is necessary to assess how they may affect the binding affinity of RBD to ACE2. Previous experiments have elucidated some roles of glycans where they, for instance, can impact antibody interactions and epitope exposure. Furthermore, different glycan types with characteristic structures can be critical for pathogen-host interaction.

Other experiments have shown that disruption of ACE2 glycosylation can impair SARS-CoV-1 viral entry into cells. However, due to structural complexity and heterogeneity of glycans, together with limited instrumental sensitivity, it is unclear how individual glycan types could distinctly modulate RBD binding. Such thorough understanding is needed to suggest more precise strategies for future experiments that seek to design effective treatments.

As Grant et al note :

Here we have generated 3D structures of glycoforms of the spike (S) glycoprotein from SARS-CoV-2, based on reported 3D structures and glycomics data for the protein produced in HEK293 cells. We also analyze structures for glycoforms representing those present in the nascent glycoproteins (prior to enzymatic modifications in the Golgi), as well as those that are commonly observed on antigens present in other viruses.

These models were subjected to molecular dynamics (MD) simulation to determine the extent to which glycan microheterogeneity impacts the antigenicity of the S glycoprotein. Lastly, we have identified peptides in the S glycoprotein that are likely to be presented in human leukocyte antigen (HLA) complexes, and discuss the role of S protein glycosylation in potentially

modulating the innate and adaptive immune response to the SARS-CoV-2 virus or to a related vaccine.

The 3D structures show that the protein surface is extensively shielded from antibody recognition by glycans, with the notable exception of the ACE2 receptor binding domain, and also that the degree of shielding is largely insensitive to the specific glycoform.

The above is an apparent reiteration of the previous observation.

Despite the relatively modest contribution of the glycans to the total molecular weight of the S trimer (17% for the HEK293 glycoform) they shield approximately 40% of the protein surface.

5.3 VARIANTS

Variants of COVID have become the driving factor[3]. We briefly summarize these variants and observe where glycans may putatively have an impact.

[3] https://www.researchgate.net/publication/349615892_COVID-19Variants_and_Vaccines

Name[4]	*Name Nextstrain*	*First Detected*	*Cases in US*	*Countries Reporting*	*Key Mutations*	*Transmissibility Rate*
B.1.1.7 Alpha	**20I/501Y.V1**	**United Kingdom**	**Y**	**70**	**69/70 deletion, 144Y deletion, N501Y, A570D, D641G, P681H**	**~50% increase**
P.1 Gamma	**20H/501Y.V3**	**Japan Brazil**	**Y**	**>4**	**E484K K417N/T N501Y D614G**	**Not determined**
B.1.351 Beta	**20H/501.V2**	**South Africa**	**Y**	**>30**	**K417N, E484K, N501Y, D614G**	**Not Determined**
B.1.526		**New York**	**Y**	**>4**	**L5F, T95I, D253G, E484K or S477N, D614G, and A701V.**	**Not Determined**
B.1.617.2 Delta		**India**	**Y**		**19R G142D 156del 157del R158G L452R T478K D614G P681N**	**High**
B.1.621 Mu		**Colombia**	**Y**		**Spike T95I Y144S Y145N R346K E484K N501Y D614G P681H D950N**	
C.37 Lambda		**South America**	**Y**		**del (S:Δ247-253, located at the N-terminal domain) G75V, T76I, D614G, L452Q, F490S, T859N**	**L452R high affinity for ACE2**
B.1.427/B.1.429 Epsilon		**California**			**I4205V in ORF1a D1183Y in ORF1b S13I W152C L452R**	

We also below focus on the N and O target changes in these variants.

Name[5]	*Alanine A*	*Serine S*	*Threonine T*	*Key Mutations*
B.1.1.7 Alpha	**From**			**69/70 deletion, 144Y deletion, N501Y, A570D, D641G, P681H**
P.1 Gamma			**To**	**E484K, K417N/T N501Y, D614G**
B.1.351 Beta				**K417N, E484K, N501Y, D614G**
B.1.526		**From**	**From**	**L5F, T95I, D253G, E484K or S477N, D614G, and A701V.**
B.1.617.2 Delta			**From**	**19R, G142D, 156del, 157del, R158G, L452R T478K, D614G, P681N**
B.1.621 Mu		**To**	**From**	**T95I, Y144S, Y145N R346K, E484K, N501Y D614G, P681H, D950N**
C.37 Lambda		**To**	**From**	**del (S:Δ247-253, located at the N-terminal domain) G75V, T76I, D614G, L452Q, F490S, T859N**
B.1.427/B.1.429 Epsilon		**From**		**I4205V in ORF1a D1183Y in ORF1b S13I, W152C, L452R**

[4] https://gvn.org/covid-19/variants-of-interest/#epsilonvariant also tracking on https://www.gisaid.org/hcov19-variants/

[5] https://gvn.org/covid-19/variants-of-interest/#epsilonvariant also tracking on https://www.gisaid.org/hcov19-variants/

6 CANCER

Glycans have been investigated for several decades as to their role in multiple cancers. We provide a brief summary of some of these malignancies.

6.1 OVERVIEW

We begin with a brief overview. As Duze and Bertozzi had noted:

Changes in glycosylation are often a hallmark of disease states. For example, cancer cells frequently display glycans at different levels or with fundamentally different structures than those observed on normal cells. This phenomenon was first described in the early 1970s, but the molecular details underlying such transformations were poorly understood.

In the past decade advances in genomics, proteomics and mass spectrometry have enabled the association of specific glycan structures with disease states. In some cases, the functional significance of disease-associated changes in glycosylation has been revealed. This review highlights changes in glycosylation associated with cancer and chronic inflammation and new therapeutic and diagnostic strategies that are based on the underlying glycobiology

The above also focuses on how experimentally to determine the specific glycan structure and location. Gupta et al have noted:

Glycosylation is the most commonly occurring post-translational modifications, and is believed to modify over 50% of all proteins. The process of glycan modification is directed by different glycosyltransferases, depending on the cell in which it is expressed. These small carbohydrate molecules consist of multiple glycan families that facilitate cell–cell interactions, protein interactions, and downstream signaling.

An alteration of several types of O-glycan core structures have been implicated in multiple cancers, largely due to differential glycosyltransferase expression or activity.

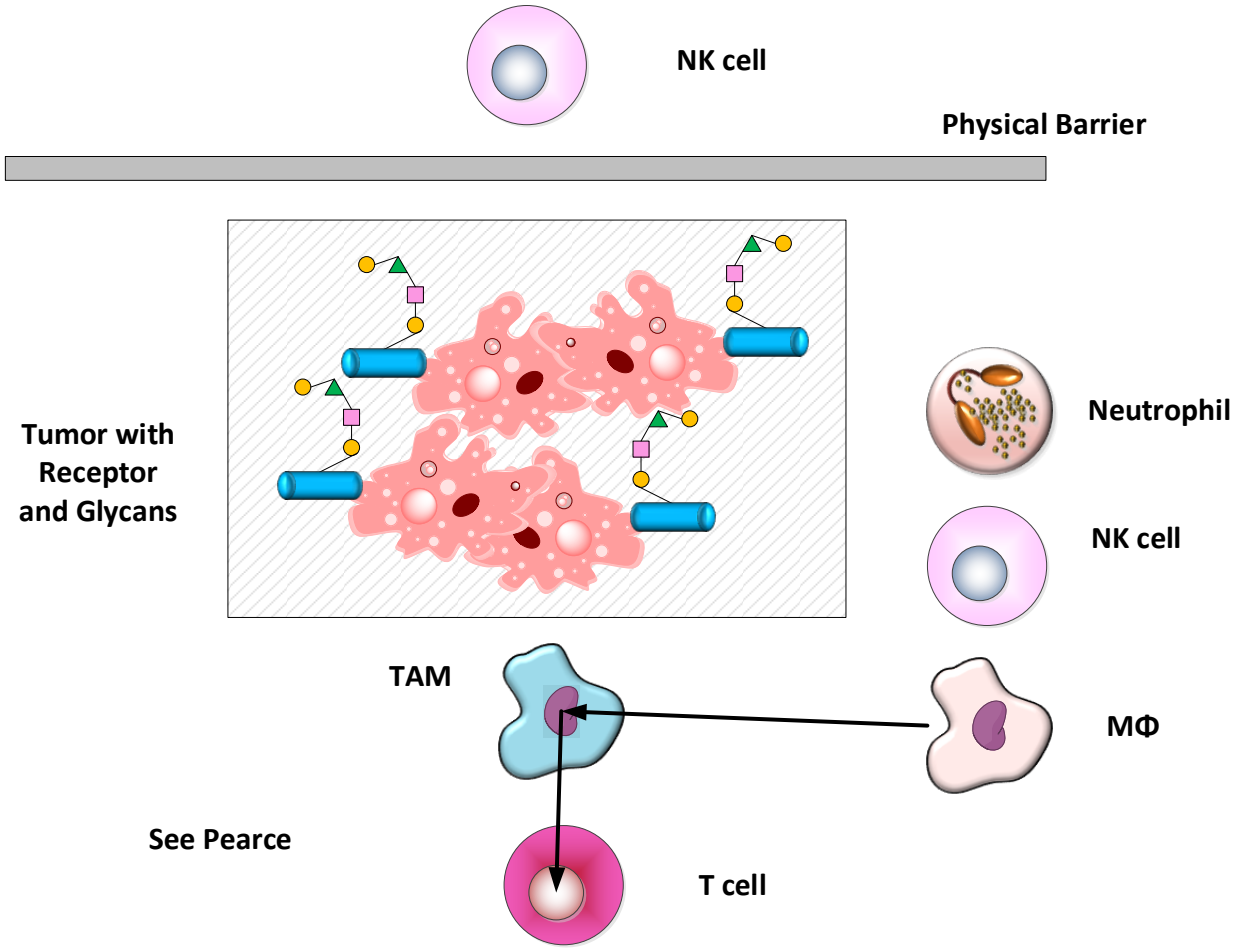
Consequently, aberrant O-linked glycosylation has been extensively demonstrated to affect biological function and protein integrity that directly result in cancer growth and progression of several diseases. Herein, we provide a comprehensive review of several initiating enzymes involved in the synthesis of O-linked glycosylation that significantly contribute to a number of different cancers

Aberrant glycan epitopes are a classic hallmark of malignant transformation, yet their full clinical potential in cancer diagnostics and therapeutics is yet to be realized. This is partly because our understanding of how these epitopes are regulated remains poorly understood. In this review cancer glycan epitopes for the major glycan classes are summarized with a focus on their biosynthesis, structure and role in cancer progression.

Their application as cancer biomarkers, in particular the more recent work on cancer glycoforms, and the advantages these offer over the glycan or protein alone are discussed.

Finally, emerging concepts which expand on the current view of the cancer glycan epitope beyond the single structure, to patterns and the whole glycocalyx, are described. These new approaches that consider the cancer glycan epitope as a glycoform, or as a pattern of many epitope structures, are providing new targets both for cancer biomarkers and therapeutics currently in development at the bench and the clinic.

As Pearce has noted in the graphic below the various interactions of glycans on tumor cells and their environment.



Pearce describes the above figure as follows:

Summary of glycan epitope interactions with immune cells. Inflammatory signaling molecules within the tumor microenvironment alter glycan processing, upregulating sialylation of glycan epitopes. Some of these sialylated epitopes are ligands for innate immune cell Siglecs which limits tumor immunosurveilance and inflammation.

The MUC1-ST glycoform has been shown to activate macrophages through Siglec-9 to generate a TAM phenotype which may potentially signal to infiltrating adaptive immune cells. The TAM phenotype is also associated with an altered secretome that includes chemokines/cytokines that feeds back into the altered glycoprocessing. Poly-N-acetyllactosamines (PLAs) expressed by malignant cells are a physical block against NK cell immunosurveillance.

In Varki et al, Chapter 47, they note:

Glycan changes in malignant cells take a variety of forms: loss of expression or excessive expression of certain glycans, increased expression of incomplete or truncated glycans, and, less commonly, the appearance of novel glycans. However, this is not simply the random consequence of disordered biosynthesis in tumor cells. It is striking that a very limited subset of changes are frequently correlated with malignant transformation and tumor progression.

Given that cancer is a "microevolutionary" process in which only the fittest cells survive, it is likely that these specific glycan changes are selected for during tumor progression.

Thus there are not a random number of changes but a limited and identifiable set of them, and furthermore they note:

Overexpression of mucins in carcinomas was first observed in classic studies of episialin (now known as MUC1) on mouse tumor cells*. Mucins are* ***large glycoproteins that carry many O-GalNAc glycans on Ser or Thr in tandem repeat regions. In normal polarized epithelium, mucins are expressed exclusively in the apical membrane, facing the lumen of a hollow organ, and soluble mucins are secreted exclusively into the lumen.***

Loss of adhesion junctions and topology in malignant epithelial cells destroys polarization, allowing soluble mucins to enter the extracellular space and the blood. The rod-like structure of mucins and their negative charge are thought to repel intercellular interactions and sterically inhibit other adhesion molecules such as cadherins and integrins from carrying out their functions.

Thus, in some instances, mucins may act as "antiadhesins" that can also promote displacement of a cell from the primary tumor during the initiation of metastasis.

Tumor mucins bearing selectin ligands facilitate several aspects of cancer progression. Evidence suggests that they might also physically block interactions between blood-borne carcinoma cells and host cytolytic cells such as natural killer cells. In addition, mucins may mask presentation of antigenic peptides by major histocompatibility complex (MHC) molecules.

The mucin excess can thus provide a basis for movement of the malignant cells. This is a fundamental element of any metastatic behavior. Furthermore they note regarding hyaluronan[6]:

[6] See Laurent and Fraser, Hyaluronan (hyaluronic acid) is a high-molecular-mass polysaccharide found in the extracellular matrix, especially of soft connective tissues. It is synthesized in the plasma membrane of fibroblasts and other cells by addition of sugars to the reducing end of the polymer, whereas the nonreducing end protrudes into the pericellular space. The polysaccharide is catabolized locally or carried by lymph to lymph nodes or the general

Many classes of malignant tumors express high levels of hyaluronan, a very large negatively charged polysaccharide composed of the repeating disaccharide unit [GlcAβ1-3GlcNAcβ1-4]. In carcinomas, hyaluronan is usually enriched in tumor-associated stroma (i.e., connective tissue, immune cells, and blood vessels). This stroma is more or less prominent depending on tumor type; for example, it is usually prominent in breast cancer.

However, hyaluronan is also localized around the tumor cell surface. In normal tissues, hyaluronan serves at least three functions, which may also contribute to tumor progression. First, it increases levels of tissue hydration, which can facilitate movement of cells through tissues. Second, it is intrinsic to the assembly of extracellular matrices through specific interactions with other macromolecules, and thus it participates in tumor cell–matrix interactions that facilitate or inhibit tumor cell survival and invasion.

Finally, hyaluronan interacts with several types of cell-surface receptors, especially CD44, which mediate or modify cell signaling pathways. These interactions, notably with alternatively spliced isoforms of CD44 that are elevated in most cancer cells, are often crucial to tumor malignancy and are a current target for novel therapies.

Furthermore, they note regarding the cancer stem cells:

Cancer stem cells, or tumor-initiating cells, are the small subpopulation of cancer cells that has the ability to initiate tumors. ***Several glycans that are specific markers for embryonic stem cells (stage-specific embryonic antigen-3 [SSEA-3], SSEA-3 with fucose [Globo H], and SSEA-4) are also expressed by cancer stem cells.*** *SSEA-1, an embryonic stem cell marker in mice, is found in cancer stem cells in human gliomas.*

Thus the expression of these glycans appear to be associated with the "stemness" of cells.

The hyaluronan receptor, CD44, is also a marker of cancer stem cells and is functionally important for their properties. Cancer stem cells are often investigated in the context of EMT.

Cells that have undergone EMT are highly similar to cancer stem cells. EMT is a critical event in tumor progression that prepares cancer cells for metastasis. It is governed by several well-defined transcription factors such as SNAIL and ZEB. Specific changes of glycan expression

circulation, from where it is cleared by the endothelial cells of the liver sinusoids. The overall turnover rate is surprisingly rapid for a connective tissue matrix component (t1/2 0.5 to a few days). Hyaluronan has been assigned various physiological functions in the intercellular matrix, e.g., in water and plasma protein homeostasis. Hyaluronan production increases in proliferating cells and the polymer may play a role in mitosis. Extensive hyaluronidase-sensitive coats have been identified around mesenchymal cells. They are either anchored firmly in the plasma membrane or bound via hyaluronan-specific binding proteins (receptors). Such receptors have now been identified on many different cells, e.g., the lymphocyte homing receptor CD 44. Interaction between a hyaluronan receptor and extracellular polysaccharide has been connected with locomotion and cell migration. Hyaluronan seems to play an important role during development and differentiation and has other cell regulatory activities. Hyaluronan has also been recognized in clinical medicine. A concentrated solution of hyaluronan (10 mg/ml) has, through its tissue protective and rheological properties, become a device in ophthalmic surgery. Analysis of serum hyaluronan is promising in the diagnosis of liver disease and various inflammatory conditions, e.g., rheumatoid arthritis. Interstitial edema caused by accumulation of hyaluronan may cause dysfunction in various organs.

are known to occur on EMT of human cancer cells, including appearance of GD1 ganglioside, and decreased expression of Gg4 and GM2 glycolipids. *Expression of known tumor-associated glycans, such as β1,6-GlcNAc branched N-glycans, SLe and SLe , is also enhanced in cancer cells undergoing EMT.*

6.2 TYPES OF CANCER

We now consider several cancers and the effect that glycans have. As Mechref et al have noted:

(1) ***Breast Cancer***

A statistically significant increase in sialylation and fucosylation of glycan structures associated with breast cancer progression was recently determined from the MALDI-MS glycomic profiles of permethylated N-glycans. Different disease stages (12, stage I; 11, stage II; 9, stage III; and 50, stage IV) were distinguished with defined trends for many glycans. This was also true for breast cancer cell lines representing invasive breast cancer (MDA-MB-231 and MDA-MB-435), non-invasive breast cancer (578T, NCI/ADR-RES, BT549 and T47D) and normal epithelial breast cells (MCF10A).

HPLC glycomic profiling with fluorescence detection coupled with exoglycosidases and MS was employed

(i) to determine glycomic changes of different ***breast cancer*** *cohorts,*

(ii) to identify women with an ***aggressive form of breast cancer*** *at an early stage and*

(iii) to identify cancer patients with higher ***circulating tumor cell counts*** *(CTCs.*

Enzymatically released N-glycans were labeled with 2-aminobenzamide prior to HPLC on a TSK gel Amide-80 or anion exchange columns.

(2) ***Colorectal Cancer***

MALDI-MS glycomic profiling of permethylated N-glycans was also recently employed to evaluate proliferating and differentiated HT-29 human colon carcinoma cells. High man structures (Hex5–9HexNAc2) and complex-type glycans (NeuAc0–4Fuc0–2Hex3–7HexNAc4–7) were observed in both proliferating and differentiated HT-29 cells.

However, the latter exhibited significantly elevated levels of four m/z values (1836, 2082, 2286 and 2327) corresponding to monosaccharide compositions of Hex3–4HexNAc4–6DeoxyHex. Additionally, four GlcNAc-terminated N-glycans constituted nearly 25% of total glycans in differentiated cells, but were almost undetectable in proliferating cells.

(3) ***Lung Cancer***

The LC with fluorescence approach, described above for breast cancer, was recently employed to analyze he N-glycans of serum samples donated by 100 lung cancer patients (20 from each stage I, II, IIIA, IIIB, and IV) and 84 age- and sex-matching disease-free donors. The levels of SLex, mono-antennary, and trisialylated glycans are significantly high in lung cancer. Also, the alteration in glycosylation of haptoglobin isolated from blood serum resembled that of the whole blood serum sample N-glycans.

A significant increase in the levels of SLex structures in squamous cell carcinoma (N=22) relative to adenocarcinoma (N=40) was also reported in this study. A correlation between glycan alterations and smoking among the samples analyzed was also determined.

***(4) Prostate Cancer**:*

MALDI-MS profiling of permethylated N-glycans, derived from 10-μl aliquots of blood sera donated by 10 disease-free individuals and 24 prostate cancer patients, suggested that fucosylation of glycan structures is generally higher in cancer samples (ANOVA test pvalue of 0.0006).

This MALDI-MS profiling allowed the detection of 50 N-glycan structures of which 12 were significantly different between the two sample sets. Six of these glycan structures were fucosylated. Ten of the 12 glycan structures were significantly higher in prostate cancer samples relative to control samples, while the other two were less abundant. These results were independently confirmed by Lebrilla and coworkers using MALDI-FT-ICR-MS.

(5) Liver Cancer

MALDI-MS profiling of permethylated N-glycans was also successful in distinguishing 73 hepatocellular carcinoma (HCC) patients, 77 age- and gender-matched cancer-free controls, and 52 chronic liver disease patients.

This study demonstrated for the first time an enhanced sensitivity and specificity when combining the distribution of several glycans. The trends of disialylated triantennary, monosialylated triantennary with terminal galactose, and trisialylated tetraantennary when considered togather was sufficient to classify HCC with 90% sensitivity and 89% specificity in an independent validation set of patients with chronic liver disease. Adjustment for chronic viral infection and other known covarites did not influence the association of the three N-glycans with HCC.

6.3 PROSTATE CANCER

Now significant work has been done on many of the above and more. We now focus on prostate cancer, PCa, and some specific targeting of glycan impact. As Scott and Munkley had noted:

Prostate cancer is the most commonly diagnosed malignancy in men, claiming over 350,000 lives worldwide annually. Current diagnosis relies on prostate-specific antigen (PSA) testing, but this misses some aggressive tumours, and leads to the overtreatment of non-harmful disease. Hence, there is an urgent unmet clinical need to identify new diagnostic and prognostic

biomarkers. As prostate cancer is a heterogeneous and multifocal disease, it is likely that multiple biomarkers will be needed to guide clinical decisions.

Fluid-based biomarkers would be ideal, and attention is now turning to minimally invasive liquid biopsies, which enable the analysis of tumour components in patient blood or urine. Effective diagnostics using liquid biopsies will require a multifaceted approach, and a recent high-profile review discussed combining multiple analytes, including changes to the tumour transcriptome, epigenome, proteome, and metabolome.

However, the concentration on genomics-based paramaters for analysing liquid biopsies is potentially missing a goldmine. Glycans have shown huge promise as disease biomarkers, and data suggests that integrating biomarkers across multi-omic platforms (including changes to the glycome) can improve the stratification of patients with prostate cancer.

A wide range of alterations to glycans have been observed in prostate cancer, including changes to PSA glycosylation, increased sialylation and core fucosylation, increased O-GlcNacylation, the emergence of cryptic and branched N-glyans, and changes to galectins and proteoglycans. In this review, we discuss the huge potential to exploit glycans as diagnostic and prognostic biomarkers for prostate cancer, and argue that the inclusion of glycans in a multi-analyte liquid biopsy test for prostate cancer will help maximise clinical utility

As Gilgunn et al note :

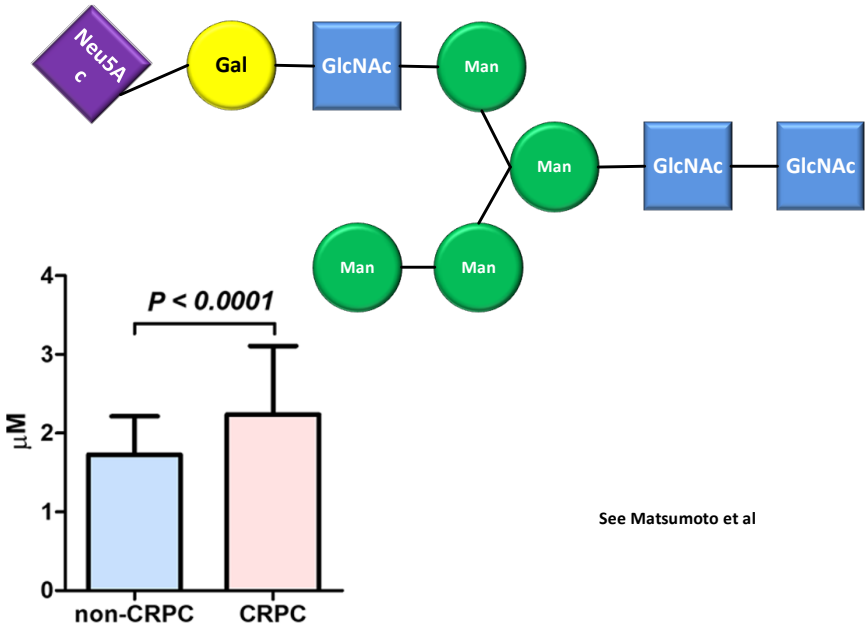
The diagnosis and treatment of prostate cancer (PCa) is a major health-care concern worldwide. This cancer can manifest itself in many distinct forms and the transition from clinically indolent PCa to the more invasive aggressive form remains poorly understood. It is now universally accepted that glycan expression patterns change with the cellular modifications that accompany the onset of tumorigenesis.

The aim of this study was to investigate if differential glycosylation patterns could distinguish between indolent, significant, and aggressive PCa. Whole serum N-glycan profiling was carried out on 117 prostate cancer patients' serum using our automated, high-throughput analysis platform for glycan-profiling which utilizes ultra-performance liquid chromatography (UPLC) to obtain high resolution separation of N-linked glycans released from the serum glycoproteins.

We observed increases in hybrid, oligomannose, and biantennary digalactosylated monosialylated glycans (M5A1G1S1, M8, and A2G2S1), bisecting glycans (A2B, A2(6)BG1) and monoantennary glycans (A1), and decreases in triantennary trigalactosylated trisialylated glycans with and without core fucose (A3G3S3 and FA3G3S3) with PCa progression from indolent through significant and aggressive disease.

These changes give us an insight into the disease pathogenesis and identify potential biomarkers for monitoring the PCa progression, however these need further confirmation studies.

From Matsumoto et al the authors present a set of glycans and their prevalence in non-CRPC and CRPC. The example below is just one example. It is interesting to see the complexity of glycans in such a profile.



See Matsumoto et al

7 OBSERVATIONS

We can now make several observations. The issue of glycans on cell receptors, such as in COVID or cancer, is quite complex. There are many as yet answered questions. As a system problem, the presence represents the existence of what we have termed a "noisy environment". Namely a receptor is not the same in all people nor most likely even in a single person. Thus therapeutics or diagnostic or prognostic measures may vary greatly due to the variation of glycan profiles. Unfortunately this just adds another layer of complexity to the cancer models and therapeutic targeting.

7.1 DOES GLYCOSYLATION BECOME MORE SIGNIFICANT IN DIABETICS WHERE GLUCOSE CONTROL IS LACKING?

Diabetics, Type I and Type 2, have excess glucose. One may then ask if this adds to the complexity of the glycan profiles we see in these patients. If we were to suppress the glucose using metformin then would we expect less malignancies? We have examined this in prostate cancer in previous papers[7]. The linkages to glyco proteins was not raised then but it now may be a point of interest.

7.2 DO GLYCANS HELP TO EXPLAIN THE VARIATION IN IMMUNOTHERAPEUTIC RESULTS BASED UPON VARIANCES IN BINDING CAPABILITIES?

In many of the biotherapeutics which target receptors we often find the 40:60 rule. Namely 40% respond and 60% do not. Admittedly these numbers may vary dramatically but our question is; do the glycans play a role, and even a significant role in this process?

7.3 CAN TARGETING OF GLYCANS BECOME AN ADDITIONAL THERAPEUTIC APPROACH?

If the glycans play a role in mitigating therapeutics, then if we know the profile of them can we then adjust the therapeutic to target the blocking glycan. Can we use polyspecific antibodies as a tool[8]?

7.4 SHOULD GLYCAN PROFILES BE PART OF GENOMIC/PROTEOMIC PROFILING OF TUMORS?

We have developed a significant set of measurements to profile tumors for diagnostic and prognostic analyses. Can we do the same with glycans? In some of the analyses discussed herein we see that such has merit.

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[7] https://www.researchgate.net/publication/351051261_Metformin_Prostate_Cancer_and_Efficacy and https://www.researchgate.net/publication/351034816_Metformin_and_Statins_in_PCa

[8] https://www.researchgate.net/publication/346245151_Poly-specific_Antibodies

7.5 DO GLYCANS INTERACT WITH EPIGENETIC FACTORS AND IF SO HOW?

We know that there are many epigenetic factors that act on DNA and thus its products. Is there as similar example of glycans and proteins to the epigenetic factors on DNA? If so, then how can we model, understand, predict, and manage them?

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